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3 Basic Principles of Analytical Supercritical Fluid Extraction

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3.1 The Development of Analytical SFE

The analytical chemist in general employs several procedural steps for the characterization of complex samples. These steps can be classified into three major tasks: sample preparation, analysis, and interpretation of the resultant data. Advances in analytical chemistry have reduced the experimental burden and time required for these task areas; however, to date, sample preparation continues to consume much of the analyst's time in the entire analysis protocol. Improved methods for sample preparation would be welcomed in the analytical laboratory as well as techniques that minimize the use of chemical reagents and their attendant disposal problems. This is particularly true in analytical protocols that require the use of toxic or carcinogenic organic solvents in the sample preparation schemes. The relatively new technique of supercritical fluid extraction (SFE) offers the analyst an alternative for preparing samples prior to analysis, that is rapid and environmentally less hazardous. This chapter will describe the basic principles involved in applying the technique for sample preparation in analytical chemistry.

3.1.1 Sample Preparation in Analytical Chemistry

An interesting review by Majors [1] summarizes the use of various techniques utilized in current sample preparation schemes. Filtration and dilution are by far the most common steps that are employed by the analyst in the preparation of his sample. Classical techniques, such as distillation and liquid-liquid extraction are time consuming and cumbersome to apply in specific cases. Often further refinement of the extract is required through such techniques as size exclusion and adsorption chromatography [2]. These low efficiency fractionation methods are gradually being replaced by miniature solid phase extraction columns. Supercritical fluid extraction is a particularly expeditious technique for the removal of non-polar to moderately polar compounds from a variety of sample matrices. However, as will be shown later, SFE can be used for the solubilization and removal of polar compounds by modifying the supercritical fluid with a cosolvent. For trace analysis, many types of compounds can be processed with SFE, since large analyte solubilities in the extraction fluid are not required.

3.1.2 Utilization of Supercritical Fluids in Analytical-Scale Extractions

Historically, the development of analytical SFE has been associated with a form of chromatography. Early SFC studies employed the principle of SFE in many of the injection devices to crudely fractionate complex mixtures [3, 4]. The research of Stahl in Germany combined SFE with thin-layer chromatography [5] for the fractionation of complex mixtures of natural products [6]. The development of practical SFC systems in the early 1980s saw the addition of micro-extraction devices which facilitate "on-line" SFE with various modes of chromatography.

Recent studies on such instruments have demonstrated that SFE has definitive advantages over conventional extraction methods. SFE has been shown to extract quantitatively environmental toxicants [7–12], pesticides [13–18], and many other compounds [19–21]. In general, extractions performed by SFE require less time than those obtained using a conventional method, such as Soxhlet extraction [8, 9, 11, 14, 19, 22]. In addition, SFE yields results having better precision, than when similar extractions are performed by the Soxhlet method [9, 11], even down to parts-per-billion level of the analyte in the sample matrix.

3.1.3 Features of Analytical SFE

Supercritical fluid extraction is a technique that employs a fluid phase having intermediate properties between a gas and liquid, to effect the solubilization of solutes. The advantages that are gained by employing SFE can be traced to the unique physical properties that these fluids possess. Compared to liquid solvents, supercritical fluids have lower viscosities and higher diffusivities, thus allowing more efficient mass transfer of solutes from sample matrices [23]. Another advantage of supercritical fluids is that their solvent power can be adjusted through mechanical compression of the extraction fluid. This feature not only permits selective extraction to be accomplished, but allows the concentration of analytes after extraction, free from any contaminating solvent. Proper choice of the extraction fluid will also allow the analyst to conduct the extraction at low temperatures, a feature which makes SFE particularly amenable to the treatment of thermally-labile substances.

The above definition encompasses the use of these fluids in the field of chemical engineering, as well as their use in analytical chemistry. It is worth noting the similarities and differences that exist in the application of SFE to these two diverse technical areas. The use of SFE in modern process engineering applications was initiated in Germany during the late 1960s to the early 1970s [24] and are well documented in the patent literature [23]. These early engineering studies showed that SFE was a viable alternative to conventional distillation and solvent extraction processes and permitted the

processing of substrates whose extraction could be adversely affected by high temperatures and the presence of solvent residuals. It is important in engineering applications of SFE to maximize the yield of the extract using a minimal expenditure of energy [25]. In addition, care must be taken to minimize the extraction pressure since this increases the costs associated with the construction of plants designed to operate at high pressures. Engineers must also be concerned with the conservation of the processing fluid, hence the recycle mode of SFE is commonly employed in many industrial separation schemes.

For the application of analytical SFE, some of the above constraints are removed. For example, the size of the sample or concentration of the target analyte for analytical purposes is usually much smaller than in the engineering case. This has two practical implications in analytical SFE. For one, the quantity of extraction gas required is considerably less than in large scale SFE, therefore an inexpensive and non-toxic fluid can be decompressed into the atmosphere after use. Secondly, for extractions involving trace quantities of analyte, much lower extraction pressures can be utilized, since large, finite solute solubilities in the supercritical fluid are not required. However, for certain analytical applications of SFE, the attainment of maximum solubility may be desired [26]; therefore higher fluid densities are needed in order to shorten the time of the extraction.

3.2 Fluid Properties in SFE

The properties of a supercritical fluid are of paramount importance when considering the selection of a fluid as an extracting agent. The analyst should strive to select a fluid that exhibits the best compromise in solubilizing the solutes of interest as well as the mass transfer characteristics required to rapidly effect the extraction of the analytes. Optimization of these two factors will assure a high flux rate of the analyte into the extracting medium, thereby saving consumption of fluid, while assuring rapid sample processing. Giddings [27] has suggested that the solvent properties of a supercritical fluid could be partitioned into a "state effect", described by the variation in the physical properties of fluid as a function of compression, and a "chemical effect" which is related to the static physical constants of the gas (fluid). We shall examine the impact of these parameters on the performance of specific fluids in the following sections.

3.2.1 Selection of the Supercritical Fluid

Two parameters which are of prime importance when considering the selection of a supercritical fluid are the critical pressure and temperature. The

Table 1. Critical constants for some common SFE solvents

Compound	Critical temperature (K)	Critical pressure (MPa)	Critical density (g/cc)
Ethylene	283.0	5.12	0.23
Carbon dioxide	304.1	7.39	0.47
Nitrous oxide	309.6	7.26	0.46
Propane	369.8	4.26	0.22
Sulfur hexafluoride	318.8	3.76	0.75
Methanol	513.4	7.99	0.27
Water	637.0	22.1	0.32
Ammonia	405.4	11.3	0.24
n-pentane	469.8	3.37	0.23

critical pressure to a first approximation determines the magnitude of fluid's solvent power in the condensed state and therefore can be used as a crude guide to match the fluid with the anticipated polarity of the compounds to be extracted. For example, ethylene has a lower critical pressure than carbon dioxide, as shown in Table 1. Based on this criterion, ethylene would not dissolve a moderately polar solute to the same extent as carbon dioxide. Likewise, fluids which exhibit higher critical pressures than carbon dioxide, are known to solubilize polar moieties at higher concentrations in the fluid phase than SC-CO_2.

The critical temperature of the fluid exerts its influence in both a theoretical and practical manner. From a practical perspective, one should consider the effect of extraction temperature on the thermal stability of the target analyte. Fluids which are characterized by high critical temperatures will require elevated extraction temperatures in order to affect extraction in the supercritical state. Conversely, fluids having sub-ambient critical temperatures may require cooling of the extractor circuit in order to promote densification of the extraction medium. Theoretically, the maximum extraction gas density is obtained by selecting an extraction temperature which is close to the critical temperature of the chosen fluid. This effect can be demonstrated by comparing the relative densities of carbon dioxide with those of nitrous oxide over the pressure range encompassing their respective critical temperatures (see Table 1) at an extraction temperature of 40 °C (313 K). At the selected extraction temperature, nitrous oxide exhibits a larger increase in density with pressure than CO_2, since its critical temperature is closer to the chosen extraction temperature [28].

The use of specific fluids can also enhance the solubility of a particular class of analytes during SFE. For example, the solubility of certain opium alkaloids in fluoroform is much larger than their recorded solubilities in SC-CO_2 over the same range of extraction pressure and temperature [29]. This trend is due to the tendency of fluoroform to exhibit a specific propensity for hydrogen bonding with alkaloid moieties in the supercritical fluid

phase. Evidence for specific adduction in supercritical fluid state has also been presented by King et al. [30], based on the computation of complexation constants from virial coefficient data for SC-CO_2-alcohol mixtures at moderate compression.

3.2.2 Unique Properties of Supercritical Fluid Carbon Dioxide

Inspection of Table 1 reveals that carbon dioxide is unique among the candidate fluids for effecting supercritical fluid extraction. Its critical temperature of 31 °C is close to room temperature, thereby permitting extractions to be carried out at low temperatures on thermally labile compounds. Modest compression of CO_2 produces a substantial change in its fluid density due to the high non-ideality exhibited by this fluid. Carbon dioxide is also non-flammable and odorless; properties which facilitate its use in a laboratory environment. Fluid carbon dioxide is also relatively inexpensive and available in satisfactory quantities. With proper ventilation, it represents little harm to the analyst.

The solubility of solutes in carbon dioxide in both the fluid and liquid phase have received considerable study in the past two decades [31]. The classical study of Francis in 1954 [32] is also worth consulting, since it qualitatively describes the solubility trends for 261 compounds in near critical CO_2. Even though supercritical carbon dioxide preferentially extracts non-polar compounds, it can exhibit an induced dipole moment [33], which enhances the extraction of moderately polar solutes into the fluid phase. Generalized solubility rules, formulated by Stahl [5], support the above observations, and indicate that the introduction of polar functional groups into the molecular structure of a compound results in a substantial reduction in solute solubility in the SC-CO_2 phase. For trace analysis, such a reduction in solubility may not be deleterious, provided that the solubility level of a compound is adequate for analytical SFE and detection.

In general, SC-CO_2 is an excellent solvent for the extraction of lipophilic solutes from a variety of sample matrices. Under the proper extraction conditions, an appreciable amount of lipid material can be solubilized in SC-CO_2, which in certain specific applications of SFE can be an advantage or disadvantage. In micro-analytical coupled SFE, the high solubility of lipids can result in the extraction of excessive amounts of solute, which may overload chromatographic columns and impact on peak resolution. In other specific cases, the high solubility for these non-polar solutes may permit the selective and easy isolation of a lipid phase associated with a sample matrix.

The basis of the latter example is illustrated in Fig. 1 where the solubility of soybean oil triglycerides is plotted as a function of extraction pressure. In this case, triglyceride solubility is a function of both extraction pressure and temperature. In the case of the 80 °C isotherm, there appears to be a minimum "threshold pressure" which must be attained to solubilize a

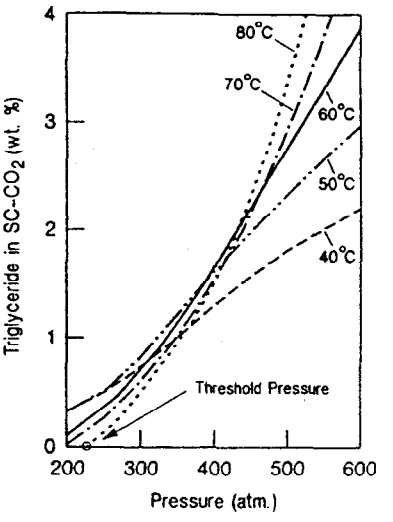


Fig. 1. Solubility of soybean oil triglycerides in SC-CO_2 as a function of pressure and temperature

"measurable" amount of lipid. Another feature in the solubility trends for triglycerides is the inversion in solubility at a particular temperature as extraction pressure is increased. This pressure interval over which the inversion in solubility occurs is called the "crossover" region, and its occurrence permits the partial fractionation of solutes [34, 35]. Beyond the crossover region, the lipid solubility increases with pressure, reaching a solubility maximum at very high pressures. The appreciable increase in lipid solubility can reach values in excess of 25% by weight, thereby permitting the rapid isolation of lipids from large samples.

3.2.3 The Use of Cosolvents in SFE

In certain cases, it becomes desirable to add a cosolvent to a supercritical fluid to enhance the solubility of an analyte in the extracting medium. Such cosolvents, also called entrainers or moderators, are usually organic solvents, that are added to the source of compressed fluid before the pump or compressor, or alternatively, to the extraction gas after it is compressed, using a high pressure liquid pump. The addition of a cosolvent to a supercritical fluid not only enhances the solubility of a analyte, but in specific cases will improve the separation factor between solutes as they are selectively partitioned into the supercritical fluid phase. Maximum separation factors are usually achieved at low solute levels in the supercritical fluid phase, a condition that is more amenable to analytical SFE than to engineering-scale SFE. The function of a cosolvent in a large scale SFE is primarily to increase the solubility of non-volatile components in the compressed gas phase and to facilitate solute separation after extraction, without resorting to a reduction in the processing pressure [36].

To date, there is no theory that can satisfactorily predict the effect of cosolvent addition on the solubility of solutes in a supercritical fluid phase. The effect of a particular cosolvent must largely be determined by experiment observation. Recently, however, a thermodynamic rationale for cosolvent selection has been proposed by Johnston and coworkers [37]. To a first approximation, the volatility enhancement of solutes into supercritical fluids is controlled by the densification of the extraction fluid; cosolvents modify the polarity of the extracting phase, and can thus improve the separation factor between solutes that differ in the number and type of functional groups in their molecular structure.

Wong and Johnston [38] have shown for similar types of solutes such as sterols, that the relative solubilities in SC-CO_2 are primarily determined by the respective vapor pressures of the sterols. The addition of a polar organic cosolvent to the SC-CO_2 in this study was found to enhance the solubility of the sterols by up to two orders of magnitude and to provide a significant enhancement of one sterol moiety over another in the supercritical fluid medium. Greater enhancements have been reported when using cosolvents which hydrogen-bond with specific solutes as demonstrated by the 620% increase in solubility reported for 2-aminobenzoic acid upon the addition of 3.5 mole% of methanol to SC-CO_2 [39].

The use of cosolvents in SFE requires that the analyst choose his extraction conditions judiciously. For example, the addition of a cosolvent to the fluid phase will change the critical point of the mixture from the one recorded for the pure supercritical fluid. Hence, it is important to recognize the magnitude of this change so as to adjust the experimental parameters commensurate with conducting an extraction in the one phase region. Likewise, the solubility of the cosolvent in the supercritical fluid is determined by the extraction temperature and pressure, therefore the quantity of the cosolvent that can be added to the fluid phase must be regulated.

Cosolvent addition to supercritical fluids may also provide some additional benefits that can improve the extraction. For example, it has been demonstrated that the addition of water to the fluid medium can change the morphology of the substrate that is being extracted and result in an improved extraction flux of a particular component, e.g. caffeine from coffee [24]. Cosolvents may also aid in the desorption of analytes from highly adsorptive sample matrices by displacing the analyte from the surface as opposed to increasing its solubility in the supercritical fluid phase. The molecular mechanism appears to be one of competitive adsorption between the cosolvent, supercritical fluid, and adsorbed analyte on the surface of the sample [12].

In recent years, very specific and novel agents have been added to various supercritical fluids to provide both enhanced and selective extraction of particular solutes. The use of an ion pairing agent such as tetrabutylammonium hydroxide has been cited for the removal of polar drugs from aqueous solution [40]. Non-polar supercritical fluids containing reverse micelles have been used to solubilize high molecular weight proteins and in-

organic salts in the compressed gas phase. For example, the amino acid, tryptophan, can be solubilized at levels 100 times more than that obtained with pure supercritical ethane by the additional of the anionic surfactant, sodium di-2-ethylhexyl sulfosuccinate, to the fluid phase [41]. Such specificity creates many interesting possibilities for the analyst over and above the results that have already been reported using conventional SFE.

3.3 Optimizing Experimental Conditions for Analytical SFE

In the practice of analytical chemistry, many analysts utilize an empirical approach to arrive at the best experimental or analysis conditions. Such an approach is made, in part, because of time limitations that are placed on the analyst and partly due to the lack of theoretical guidelines which can be utilized when analyzing complex samples. Many theoretical approaches have been developed for predicting the solubility of solutes in supercritical fluid media [42] but they are of limited value to the practicing analyst because of computational time required and the lack of physical property data on the target analytes, solvents, etc. Complicating the problem is the influence of the sample matrix which may have a synergistic or retardative effect on the recovery of solutes.

3.3.1 Objectives of the Extraction

To chose the optimal conditions for performing SFE, the analyst must define his analytical objective, since for many applications, SFE is not a highly discriminative technique. In fact, SFE rarely allows the isolation of one specific analyte to the exclusion of other co-extracted moieties. A crude form of fractionation can be accomplished by SFE [43] by simply varying either the extraction pressure or temperature. Such a method yields positive results only when there are significant differences in the molecular weight or polarity between the components that are being extracted.

An example of this approach is illustrated in Fig. 2 where SC-CO_2 has been used to fractionate the components in grapefruit oil during an on-line SFE/SFC extraction. Utilizing a fluid density of 0.18 g/cm^3 for the extraction, allows the isolation of specific components from the oil matrix. This low-pressure or density "skimming" of selected compounds can be used to advantage to analyze specific components in a complex matrix [14]. The use of higher extraction densities removes additional compounds; however, inspection of Fig. 2 reveals there are common components that are extracted at each fluid density. The lack of selective extraction can tax the chromato-

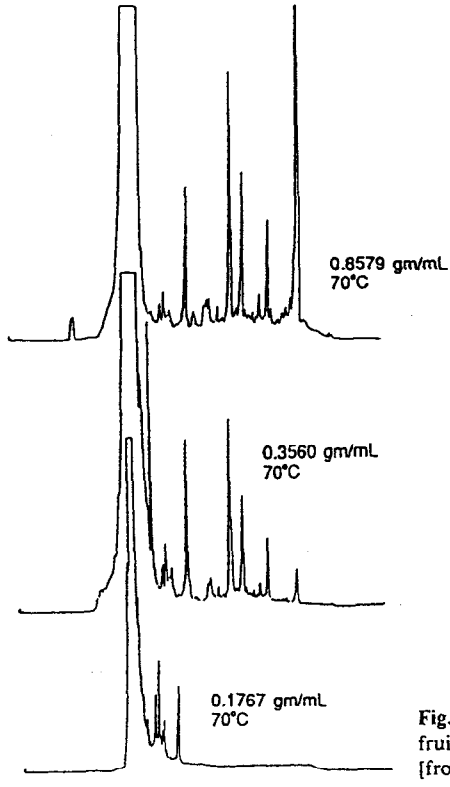


Fig. 2. SFE-SFC profiles of cold pressed grapefruit oil obtained at different extraction densities [from Ref. 82]

graphic resolving capabilities of an on-line extraction system, particularly when the target analytes are minor components in the SFE.

Total or complete extraction of the sample matrix by SFE requires that the extraction time be optimized [44]. Fractionation of a mixture by SFE shows not only a dependence on the experimental pressure and temperature, but time as well. The observed molecular discrimination that occurs as a function of time is often molecular weight dependent. Hence, for an integral off-line extraction, the initial samples taken on a sequential basis will be enriched in lower molecular weight components. The time required to complete a total extraction of the sample will be dependent on the mass of the sample taken for extraction and the level of extractable material (by SFE) in the sample. Total SFE of lipid phases from samples by SC-CO_2 has been reported and can be accomplished quite easily by off-line SFE [26].

The above citations are but two examples of the versatility of SFE. To apply SFE properly, the analyst must become familiar with several critical parameters that have impact on the extraction. These are discussed at length in the next section.

3.3.2 Critical Parameters Pertinent to SFE

There are several parameters which are of key importance in conducting an extraction by SFE. These are 1) the pressure (or density) at which the desired solute becomes miscible with the supercritical fluid phase, 2) the condition for attaining maximum solute solubility in the extraction fluid, and if available, 3) the physical and chemical properties of the solutes that are to be extracted. In general, knowledge of the miscibility pressure and solubility maxima for a given solute/fluid system will define the potential range of pressures for fractionating the extracted components [13].

The miscibility pressure for a given solute-solvent pair remains a somewhat ill-defined concept, since it depends on the technique that is used to measure the solute's solubility in the supercritical fluid. Giddings [28] defined the concept of the "threshold pressure" based on the ability of a flame ionization detector to detect a solute in a flowing stream of supercritical fluid. King [45] has noted that it is possible to employ a range of techniques for determining a solute's threshold pressure in a supercritical fluid, that vary in sensitivity over a range of 10^9! For the analyst, it is most important to have a knowledge of the relative miscibility pressures of different compounds, since their relative magnitudes will determine the feasibility of separating components based on this principle. Unfortunately, many compounds exhibit small differences in their respective miscibility pressures, so that selective SFE of individual compounds proves difficult in practice, due to the high precision required in regulating the extraction pressure and the commensurate small solute solubilities that exist under these conditions.

Knowledge of the pressure required for achieving maximum solubility of the solute in the supercritical fluid phase has several important implications in analytical SFE. Extractions conducted under these conditions can shorten the extraction time and also permit the processing of larger samples for analysis. Unfortunately, there is usually a loss in extraction selectivity under these conditions and the compression requirements can be quite high. For example, the removal of lipid phases from natural products is best affected at pressures in excess of 69 MPa, where infinite miscibility of triglycerides in SC-SO_2 is attained [46]. Extractions conducted at these conditions can remove grams of lipid from a sample within fifteen minutes [26].

The physical properties of the solute can also play a role in SFE. Particularly germane are the compound's melting point and vapor pressure. Threshold pressures have been shown to be dependent on the melting point of a compound and it has been observed that compounds with melting points in excess of 350 °C are not readily solubilized in dense CO_2 [47]. SFE is also more easily affected when the extraction is conducted at a temperature above the compound's melting point, since the solid's cohesional energy is reduced. The effect of a solute's vapor pressure on the enhancement factor in SFE has been described theoretically [48] and it has been generally observed, that beyond a certain pressure, a compound's solubility

in a supercritical fluid increases with increasing temperature [49]. This latter trend is due to the substantial reduction in the solute's cohesive energy density with increasing extraction temperature which outweighs the commensurate loss of solvent power in the extracting fluid.

3.3.3 A Theoretical Approach for Optimizing SFE

As noted previously, analysts are prone to ignore theoretical approaches for optimizing SFE, due to the lack of time or complexity of the theory. In the course of our research, we have developed a relative simple method, based on combining the solubility parameter theory with the Flory-Huggins interaction parameter concept, which explains many of the salient features of SFE. The data required by the above theory consists of fluid and solute critical or reduced property data and solubility parameters. Such data, if unavailable, can be estimated from corresponding states theory [50], group contribution methods [51], or nomographs [52].

The key equations utilized in this approach are

$$\chi = \chi_H + \chi_S = \frac{\bar{V}_1}{RT}(\delta_1 - \delta_2)^2 + \chi_S \tag{1}$$

where χ is the total interaction parameter χ_H, χ_S are the enthalpic and entropic interaction parameters, respectively; δ_1, δ_2 are the solubility parameters of the supercritical fluid and solute, respectively, and $\bar{V}$, is the molar volume of the fluid. The parameters δ_1, δ_2 and $\bar{V}_1$ are dependent on pressure and temperature and this factor must be taken into account when computing their values. The solubility parameter of the supercritical fluid, δ_1, is calculated by the method of Giddings [27] as

$$\delta_1 = 1.25\, P_c^{1/2}(\varrho_r/\varrho_{r_1\,\text{liquid}}) \tag{2}$$

where P_c is the critical pressure of the fluid, ϱ_r is the reduced density of the supercritical fluid, and $\varrho_{r_1\,\text{liquid}}$ is the reduced density of the near-liquid fluid at conditions approaching infinite compression.

Equation 1 can be rearranged to reveal its functional dependence on pressure. In this form, assuming a constant value for χ_S, plots of χ_H versus pressure are hyperbolic, with the a minimum occurring at a pressure when δ_1 equals δ_2. At this condition, maximum solubility of the solute occurs in the supercritical fluid. A common misconception in applying the above theory to SFE is that the extraction conditions must be chosen to achieve maximum solute solubility in the extracting fluid. However, for certain applications of SFE, such as the extraction of trace quantities of analyte, much lower pressures and δ_1 will suffice. In addition, it is not necessary for the respective solubility parameters of the fluid and solute, δ_1 and δ_2, to match, to achieve an effective SFE. Figure 3 illustrates this principle with a solubility parameter scale encompassing the values for a number of polar

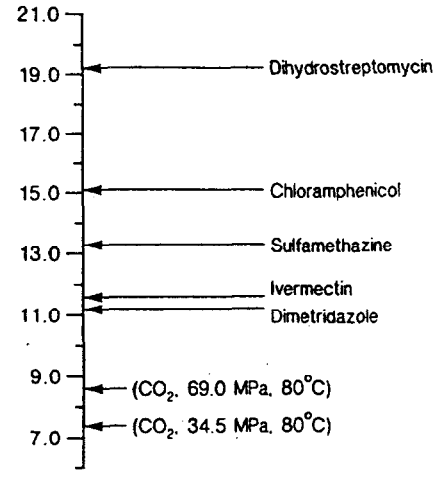


Fig. 3. Solubility parameter scale for SC-CO_2 at specified pressures and assorted drug analytes

compounds (drugs) and SC-CO_2 at two levels of compression. The obvious mismatch in solubility parameters of the drugs and extracting fluid does not mean that trace quantities of drugs, such as sulfamethazine or chloramphenicol, cannot be extracted into SC-CO_2. However, significant differences in δ_1 and δ_2 indicate a low solubility of the solute in the extracting fluid [13], and this may limit the quantitative recovery of such moieties in SC-CO_2.

Assessment of the miscibility pressure of the solute in the supercritical fluid phase can be approximated by employing the Flory [53] critical interaction parameter concept. Here, the critical interaction parameter, χ_c, is given by Eq. 3 as

$$\chi_c = (I+x^{1/2})^2/2x \tag{3}$$

where $x = \bar{V}_2/\bar{V}_1$ and $\bar{V}_2$ is the molar volume of the solute. Since χ_c is a function of $\bar{V}_1$ and $\bar{V}_2$, χ_c will have a weak dependence on pressure. Plots of χ and χ_c as a function of fluid pressure will show a common intercept, whose value on the pressure axis corresponds to the solute's miscibility pressure in the supercritical fluid. The above approach has been shown to accurately predict the miscibility of the pesticide, DDT, with SC-CO_2 [13] and the pressure range which is applicable for the fractionation of oligomers in SFC.

3.4 The Relevance of SFC-Derived Data to Analytical SFE

As a technique, supercritical fluid chromatography can be used independently of SFE, as attested in the other chapters of this book. However, in

many cases, SFC can assist in the development of an analytical SFE method. The relevance of SFC-derived data goes beyond its use as a characterization tool for SFE-derived extracts, either on an analytical scale [40, 54–63] or for monitoring pilot plant SFE processes [64]. Two generic areas of application are worth noting: (1) the utilization of SFC measurements to derive data relevant to analytical SFE, and (2) the implications of such measurements to the analytical chemist in SFE method development.

3.4.1 Relevant Measurements by SFC

The application of SFC for the measurement of physicochemical data has been documented in several review articles over the past decade [65, 66]. In this section, we shall primarily be concerned with noting the relevance of such data to analytical SFE. Table 2 tabulates some of the parameters which can be derived from various SFC experiments.

Data such as diffusion and virial coefficients are derived from solute peak broadening experiments or the pressure dependence of retention constants, respectively [67, 68]. Although these parameters are of value in optimizing a SFE, they provide no immediate information to the analyst that is likely to help in solving the analytical problem at hand. The determination of sorption isotherms or the measurement of solute solubilities in supercritical fluids by chromatographic methods require the construction of specialized apparatus [69] and employ different forms of chromatography [45]. Such experiments require excessive time and knowledge that may not be available to the analytical chemist.

An example of a useful and rapid SFC experiment for the analytical chemist is depicted in Fig. 4, where the miscibility pressure as a function of temperature has been determined for the pesticide, malathion, in SC-CO_2. The data were taken using a supercritical fluid chromatograph equipped with a nitrogen-phosphorus detector, which permitted the detection of trace levels of the organophosphorus pesticide in the column eluent. The instrument was operated at various combinations of pressure and temperature that were sufficient to just elute and detect a trace quantity of the injected pesticide. Therefore, at a temperature of 40 °C, a CO_2 pressure of approximately 75 atmospheres was required to solubilize a small quantity of

Table 2. Parameters derived from SFC experiments

Diffusion coefficients
Sorption isotherms
Phase distribution constants
Solubility measurements
Critical loci
Solute partial molar volumes
Virial coefficients

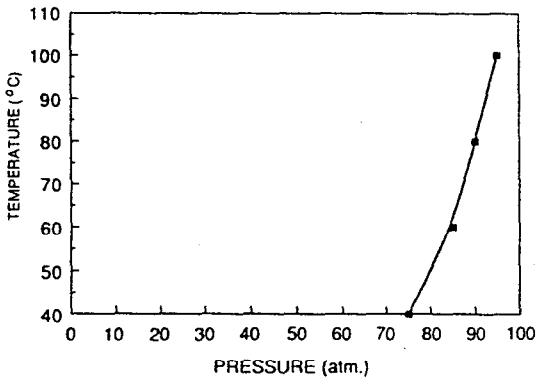


Fig. 4. Miscibility pressure for malathion in SC-CO_2 as a function of temperature and pressure as determined by SFC-nitrogen/phosphorus detector

malathion into the supercritical fluid phase. Note that an increase in temperature required a higher fluid pressure in order to initially solubilize the pesticide into the dense carbon dioxide phase. The data derived from the above experiment provides the analyst with information concerning the minimum extraction pressure required to extract trace levels of the pesticide.

Similarly, King and coworkers [70] have utilized elution SFC to ascertain the ease of extraction of soybean oil volatiles from various sorbents. In these experiments, simple columns were constructed containing the sorbent material and inserted into the SFC. Elution pulse chromatographic experiments were performed at various extraction conditions to determine the pressure and temperature required to desorb the solutes from the adsorbent column. The derived data proved of value not only in choosing the proper sorbent and conditions for stripping the volatiles from a large scale SFE process stream, but in the design of a side stream sampling device to collect the solutes under elevated pressures and temperatures.

3.4.2 Implications for SFE

Other uses for SFC in the development of SFE methods are more indirect then those cited above, but just as beneficial to the analytical chemist. For example, the SFC literature is an excellent source for identifying compounds that are most likely to be amenable to SFE [71–73]. To illustrate this point, the work of Fields and Grolimund [74] is worth citing, since it demonstrates which amine solutes can be solvated in SC-CO_2 using capillary SFC. Similar experiments can be performed quite easily by analyst, since they provide instant verification on the extractability of a particular compound. Obviously, if the compound in question does not chromatograph successfully, then the analyst should consider modifying the conditions for SFC,

or perhaps explore the possibility of choosing an alternative extraction fluid or cosolvent.

SFC retention data has also been shown to have a predictive value in determining the correct cosolvent concentration that is required for the extraction of thermally labile herbicides from a variety of sample matrices [75]. In this case, elution order was found to correlate with relative extraction efficiency of the herbicides from various soils. Examination of other chromatographically-derived parameters, such as solute capacity factor [76], should aid the analyst in the selection of extraction conditions or for the choice of cosolvents [77]. It should be recognized however that solute chromatographic mobility through columns may not always mimic the environment under which the SFE is performed, and the analyst should exert caution in correlating the results from the two techniques.

3.5 The Practice of Analytical SFE

The practice of SFE requires that the analyst construct or purchase suitable equipment for conducting the extraction and establish the proper experimental conditions required for the development of the analytical method. A key question facing the analyst will be whether to conduct the extraction in the "off-line" mode, or to combine the extraction step with another analytical technique, thereby facilitating a "on-line" mode of extraction. Utilization of the off-line mode of SFE offers many advantages to the analyst who is inexperienced in SFE. On-line SFE requires that the analyst understand and control more then one technique simultaneously, and therefore may not be the best starting point for the novice to SFE. In the following sections we shall discuss the equipment and experimental variables that are required to conduct both modes of SFE.

3.5.1 Equipment Requirements

Both off-line and on-line modes of SFE share a common base in the equipment that is used to facilitate the extraction. Generically speaking, an analytical supercritical fluid extractor consists of a source of fluid, a fluid delivery module, an extraction cell, a backpressure regulating device, and a collector for trapping the extract after SFE. The fluid is usually supplied in high pressure gas cylinders, which can be equipped with inductor tubes, if the fluid is to be pumped to into the SFE apparatus. In some cases, the fluid tank pressure has been found to be sufficient for performing SFE of trace levels of toxicants from various sample matrices [14].

There are a plethora of fluid delivery devices ranging from high pressure diaphragm compressors, to gas booster pumps, or reciprocating piston

pumps, and syringe pumps. Each device has its own merits, but there are some general principles worth considering in selecting the fluid delivery module. Many of the pumps used for delivery the fluid in analytical SFE are modified high performance liquid chromatography (HPLC) pumps and require an external cooling source to assure liquefaction of the fluid. Such cooling is critical to the performance of plunger-based pumps to avoid cavitation due to the introduction of a two phase fluid mixture at the pump head. Syringe delivery pumps that are used for both SFE and SFC, also require a source of coolant for effective operation. It is well known in engineering-scale SFE, that liquid pumps are thermodynamically more efficient than compressors for delivery of the fluid to the extractor. Delivery of a liquified gas also permits easy blending of cosolvents with the principal fluid. The merits of using compressor technology mainly lie in the increased fluid flow capacity that can be obtained with relatively inexpensive equipment and the elimination of the external cooling requirement cited above for pumping modules. Compressor based fluid delivery devices may also require the use of ballasts to dampen to pneumatic pulse from the compression stroke and its effect on the flow of the fluid.

Extraction calls have been fabricated out of a variety of materials, but most cells consist of a tubular metal cavity with associated compression fittings. Many of the reported on-line SFE studies have incorporated modified HPLC column guard cartridges as sample holders. Such cells have finite lifetimes, principally due to the development of leaks around the sealing components of the cell. Alternative sealing methods exist that are based on high pressure coned-and-threaded connections [26] which yield very long service lifetimes. The size of the cell should be scaled to avoid excessive void volume, which in turn reduces the amount of fluid required for the extraction. Incorporating diffusers at both the entrance and exit of the extraction cell will assure that the fluid stream makes contact with the entire sample during the extraction.

Devices used for regulating the extraction pressure on the extractor cell have ranged from narrow fused silica capillary tubing, to bona fide backpressure regulators, or micrometering valves having adjustable flow orifices. The selection of the device is governed by fluid flow rate desired through the extraction cell and upstream pressure desired in the extractor. The use of a silica capillary as a rate limiting orifice requires that the analyst adjust the flow rate through the extraction cell by varying the length or internal diameter of the capillary tubing. Such a procedure is cumbersome compared to using a micrometering valve or a accurate backpressure regulator in conjunction with a precise fluid delivery pump [26]. Another viable option is to use a micrometering valve for flow control into the extraction cell and a backpressure regulator at the exit of the cell.

The need to isolate the extract after SFE has fostered a number of ingenious collection schemes. A cardinal principle in selecting a collection device is to use a vessel with sufficient volume to insure adequate collection efficiency of the extracted solutes. Utilization of too small a collection

volume can result in entrainment of the extracted solutes in the expanding fluid stream. Many investigators have incorporated packing in the collector to induce precipitation or applied cooling to assure that an adequate phase separation takes place. Collector sizes have ranged from flasks containing several hundred milliliters [60] to small, 1–2 milliliter, vials [7]. The Joule-Thomson expansion which accompanies the expansion of the fluid to ambient conditions can be used to advantage when collecting the extracted sample in volatile solvents [78]. The cooling power of the expanding extraction fluid prevents evaporation of the collection solvent which absorbs the target analyte. Collection in enclosed vessels equipped with sampling tubes can be used to advantage when sequential sampling during the extraction is desired [26]. Sorbent trapping, or "accumulators" have also been utilized for collecting extracted analytes and this method may be preferred when the fluid stream contains volatile analytes.

A discussion of the large variety of extractors is beyond the scope of this chapter. Designs range from very simplistic, single sample off-line extractors, to complex "on-line" versions which incorporate syringe pumps for both SFE and SFC and utilize cryocooling or retention gaps with sophisticated valving to transfer the solute between the extraction and chromatography module. A very simple off-line extractor is portrayed in Fig. 5 and will serve as an example to convey how a supercritical fluid extractor is assembled. In this example, liquified carbon dioxide is supplied to a syringe pump through a high pressure cylinder equipped with a inductor tube. The liquified gas is converted to the supercritical fluid state prior to filling

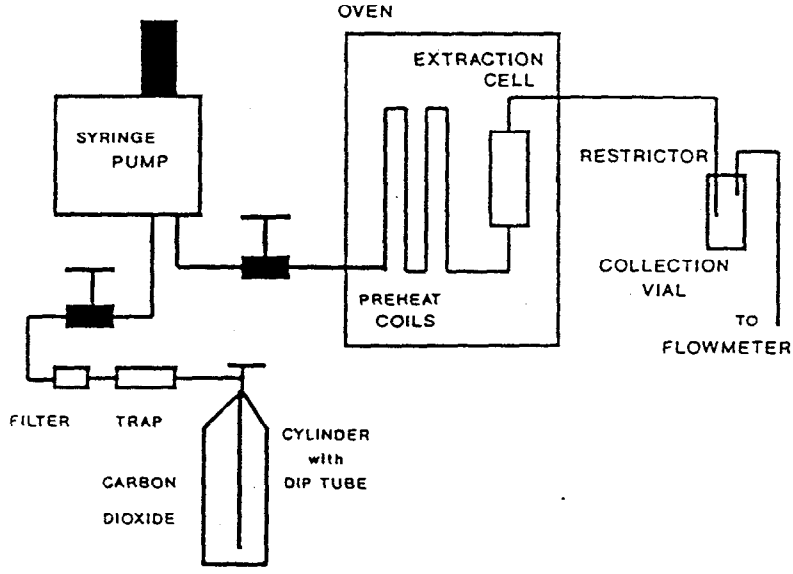


Fig. 5. Schematic of an off-line supercritical fluid extractor

the extraction vessel through the use of a heat exchange coil. The back-pressure regulating device in this case is a narrow bore tube which restricts the flow and increases pressure upstream. Collection of the extracted material is facilitated in a vial. Such a system may also include a flowmeter or gas totalizer to assess fluid flow or passage.

3.5.2 Experimental Considerations in Analytical SFE

The analyst must consider a number of experimental details to achieve a successful SFE. For example, the effect of temperature on the physical state of the supercritical fluid and the resulting phase equilibria is particularly important in executing SFE. It is critical that the SFE system be adequately heat traced so that a single phase is maintained prior to decompression and collection of the extracted solutes. Failure to maintain isothermal conditions in the extraction cell can lead to variable results; however, such conditions are not required throughout the entire system, provided that analyte precipitation is avoided prior to its capture from the fluid stream. It is important to preheat the supercritical fluid prior to its introduction to the extraction chamber. This step can be accomplished through the use of a coiled fluid introduction tube or a more formal heat exchanger arrangement. Likewise, heating of the decompression valves or regulators downstream from the extraction cell is standard practice, in order to assure that precipitation of the extracted analytes does not occur in the valve orifices. Such a situation could cause cessation of fluid flow, due to the cooling effect accompanying the expansion of the supercritical fluid to ambient conditions. Surprisingly, a number of commercial SFE devices have been constructed which violate some of the above criteria.

The materials which comprise extraction equipment are usually checked with respect to their pressure ratings, but frequent physical inspection of the cell and its components should be standard practice. Cell and associated tubing or valves should be inert with respect to the extraction conditions. Sample matrices which contain water should be extracted using rust resistant alloys, since even carbon dioxide can be regarded as a weak acid anhydride. A more critical component are the polymeric materials used as o-rings and seals in valve stems and associated high pressure fittings. Such elastomers may undergo expansion under compression or be solvated by contact with the supercritical fluid media. This phenomena may lead to leaks in the extraction system or extraction of unwanted contaminants into the isolated extract.

Another critical parameter in performing SFE is the design and orientation of the extraction cell. The size of the sample cell should be sufficient to address the analysis problem. As noted previously, many in-line extractors coupled to supercritical fluid or gas chromatographs utilize cells ranging in volume from 1 – 10 milliliters. Cells of this capacity are particularly appropriate for cases where the available sample size is small, however they

are of insufficient capacity for other types of analyses. Sizing of the extractor vessel should never be based on the available fluid delivery system, but should be scaled to a level that will allow extractions to be performed on a sample size that accurately reflects the matrix being analyzed. Large extraction cells along with ancillary equipment have been described for use in performing off-line analytical assays [26].

Cell geometries have tended to be cylindrical in deference to the variety of high pressure tubing utilized in constructing extraction cells. Other designs have been reported which permit recirculation of the extracting fluid through the sample matrix [79] or transmission of the supercritical gas through a aqueous matrix [40]. Using identical cells, Andersen et al. [80] have noted differences in extraction efficiency depending on whether the extraction cell was held in a vertical versus horizontal position. In certain cases, a physical inversion of the sample and the extracting fluid may occur within the cell due to change of density of the extracting medium with respect to the sample. Regardless of the extraction cell's spatial arrangement, it is important to have the vessel well packed with the sample of interest and to provide sufficient fluid to assure that the extraction is complete.

The kinetics of a SFE parallels trends found in liquid-liquid extraction. In general, quasi-equilibrium extraction gives way to diffusional-limited extraction kinetics in the latter stage of the SFE. Factors which impact on the rate of completion of the extraction, such as the sample matrix, will be discussed in the next section. From a practical perspective, it is important to ascertain how much fluid is required to complete the SFE. This parameter may be expressed as a volume of fluid at either supercritical fluid or ambient conditions, or more preferably the mass of extraction fluid. Some researchers [81] advocate the use of cell volumes as a measure of the fluid required to complete the extraction. This is a questionable practice since the contents of the cell can be compressed during the course of the extraction, thereby changing the void volume of the cell.

In addition, changes in the flow rate of the fluid can occur during the extraction. These variations in fluid flow can be measured with the aid of a mass flowmeter or flow totalizer unit. The completeness of a off-line extraction can be monitored by taking intermittent samples for analysis. For extraction systems in-line with a form of chromatography, simply running a second extraction will usually suffice to indicate whether the analyte of interest has been completely removed.

The isolation of the extract, either from a on- or off-line SFE system requires that certain precautions be taken. For instance, the temperature chosen for enacting cryofocusing can introduce a bias into the sample that is trapped in the tee or retention gap [82]. This will change the distribution of the components in the resultant extract and hence can be used as a aid in fractionating the total extract. Similarly, sorbent-filled columns can be used to fractionate or isolate specific analytes from the fluid stream after extraction, either at ambient or pressurized conditions. In the former case,

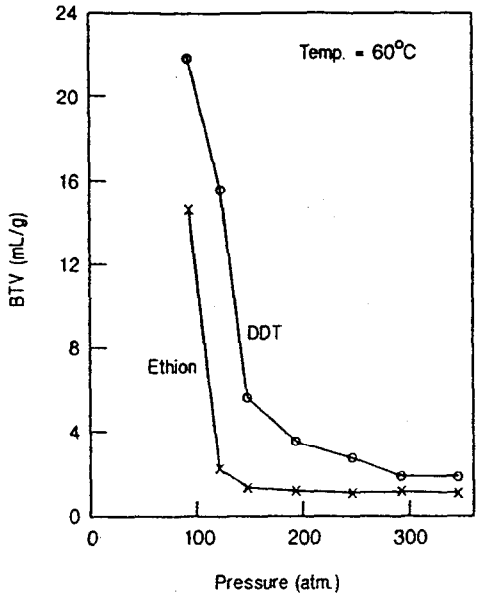


Fig. 6. Breakthrough volumes (BTV) for chlorinated pesticides on alumina as a function of pressure as determined by elution pulse chromatography

it is important to remember that a supercritical fluid will undergo close to a thousand-fold expansion upon decompression onto a "accumulator" cartridge, requiring that a adequate sorbent be available to capture the targent analyte without breakthrough occurring from the trapping cartridge. Similar considerations also apply when collecting or fractionating analytes on sorbents under elevated pressure conditions. As shown in Fig. 6, the breakthrough volume for two pesticides, DDT and ethion, on the sorbent, alumina, decreases with increasing pressure of CO_2. Therefore, capture of two pesticides is best undertaken at pressures below 100 atmospheres, while recovery of the analytes can be rapidly accomplished at pressures above 200 atmospheres. It should be noted that selective desorption of specific analyte classes from particular sorbents can be affected by changing the nature of supercritical fluid as reported by Levy et al. [83] and by Alexandrou and Pawliszyn [9].

3.6 Sample Matrix Effects in SFE

The nature of the sample matrix can have a profound effect on the results that are obtained with SFE. Unfortunately, a knowledge of analyte solubilities in supercritical fluids does not always allow a prediction to be made as to the effectiveness of SFE for extracting a particular matrix [84]. Extraction of real sample matrices, such as soils or biological tissue, should

be carried out experimentally, rather then depending on theory or results obtained on neat analytes. In this section we shall examine the factors in the sample matrix which influence the results obtained via SFE.

3.6.1 Physical Matrix Effects

The physical morphology of the substrate undergoing SFE can have a pronounced influence on the efficiency of the extraction and the rate at which it is conducted. In general, the smaller the particle size of the substrate, the more rapid and complete the extraction will be. This effect is largely due to the shorter internal diffusional path lengths over which the extracted solutes must travel to reach the bulk fluid phase. Studies [85] have shown that the geometric size of the matrix particles can influence the speed and completeness with which a SFE can be conducted. As in solid-liquid extraction, an increase in a matrix's porosity will generally promote a more efficient and rapid SFE.

The leaching of a large amount of solute(s) from a sample matrix can weaken the internal structure of the substrate, leading to comminution of the matrix within the extraction cell. A deleterious artifact of this process can be potential plugging of the sample matrix in the extractor. This condition can be partly alleviated by reversing the flow of the extraction gas via a tandem arrangement of valves or by reducing the flow rate of fluid through the matrix.

3.6.2 Chemical Changes in the Sample Matrix

The chemical composition of the sample matrix can have either an enhancing or retarding effect on the results that are obtained with SFE. One of the major parameters that influences the composition of the supercritical fluid extract is the moisture level in the sample matrix. For example, aroma oils from tobacco products are preferentially removed in the absence of moisture, while the presence of water is required for the extraction of alkaloids from the tobacco matrix [86].

The effect of moisture on the SFE of analytes from biological tissues has been a point of controversy for some time among researchers. However, it appears that partial dehydration of the sample matrix will allow a more rapid SFE to be performed. This is due to the fact that highly hydrophilic matrices inhibit contact between the supercritical fluid and the target analytes.

King and coworkers [26] have demonstrated that the removal of water can have a dramatic effect on the recovery of lipid moieties from meat products. Similar trends have been noted by other research groups concerned with the removal of pigments from krill [87] and drugs from body organs

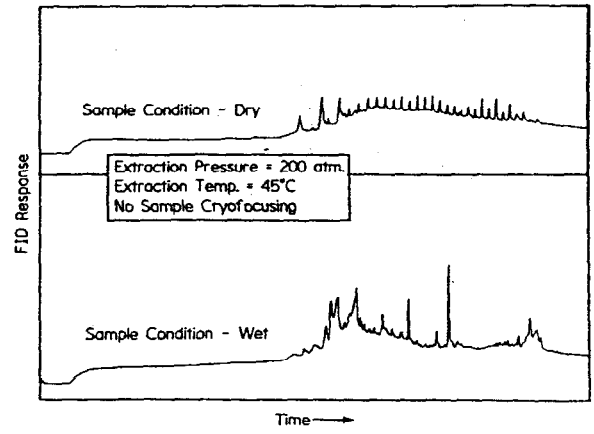


Fig. 7. SFE-SFC profiles of an aquifer solids sample before and after freeze drying of the sample

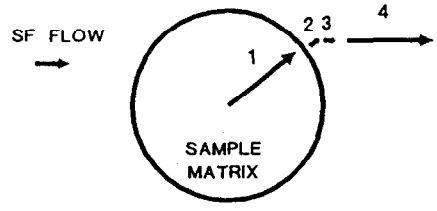
[62]. However, in some specific cases, the presence of water may actually aid in the recovery of the target analyte by acting as a "internal cosolvent". For this reason, some analysts actually spike the sample matrix with water before performing SFE. A rather dramatic example of the effect of water in the sample matrix on the results of a SFE are shown in Fig. 7. Inspection of the resultant chromatograms shows that the two profiles are different with respect to the number of components and their relative distribution in the sample. These observed differences suggest that the presence of water in the soil matrix may promote the extraction of some components into the fluid phase relative to those obtained from a dry sample.

3.6.3 Impact of Matrix on Extraction Kinetics

The rate of removal of a solute from a matrix using a SFE is a function of its solubility in the fluid media and the rate of mass transport of the solute out of the sample matrix. Rate limiting kinetics can adversely impact on the rapid extraction of an analyte despite favorable its solubility characteristics in the supercritical fluid medium. This situation often occurs when the analyst is trying to isolate a target analyte from a sample matrix, such as a sorbent or a soil.

It is useful to invoke a simple extraction model for the SFE of an analyte from a single particle to visualize the rate inhibiting mechanisms which impact on the extraction. As shown in Fig. 8, there are four major mass transport mechanisms to consider:

- analyte diffusion through the internal volume of the sample
- surface desorption of the analyte



1 = Diffusion through matrix
2 = Desorption from surface
3 = Diffusion through 'SF' surface film
4 = Transport in SF flow

Fig. 8. Mass transport steps for the SFE of an analyte from a porous matrix particle

- diffusion of the analyte through a surface boundary layer
- transport in the bulk supercritical fluid phase

If the rate determining step (RDS) is intraparticle diffusion, then the rate of extraction will be a function of the particle size of the sample matrix. It should be recognized that some matrices when exposed to supercritical fluids swell, thereby facilitating the mass transport of the analyte from within a sample matrix [88]. An excellent example of this principle is the observation that polymeric films are plasticized by supercritical gases [89]. This undoubtably contributes to the recent success achieved by analytical chemists in applying SFE for the analysis of additives in plastics [90].

Surface desorption of an analyte by a supercritical fluid is an important step in SFE for many sample types. For certain analyte-matrix combinations, the "solvent power" of the supercritical fluid alone will not suffice to assure a rapid or complete extraction. Studies on the regeneration of adsorbents [91, 92] have shown that many compounds are note completely recovered with neat supercritical fluids and that desorption times are prohibitively long. The use of a cosolvent, such as water or methanol, will frequently accelerate the desorption of an analyte from the surface of the sample matrix. Wheeler and McNally [15] have shown that extraction efficiencies of herbicides from soils can be increased by direct addition of microliter quantities of ethanol or methanol to the sample before commencement of extraction.

Diffusion of the analyte through a surface boundary layer may also kinetically influence analyte extraction. As noted by King [93] and Parcher [69], many solid samples will promote condensation of a surface layer of the dense extraction fluid at the fluid-solid interface. The density of the adsorbed surface film will partly depend on the pressure applied to the supercritical fluid and the affinity of the sample matrix for the fluid. The development of a condensed fluid film at the surface of the sample matrix can aid in the recovery of certain analytes through competitive adsorption at the sample interface [93] as well as inhibit the transport of the analyte into the fluid phase. The kinetics of transport through a rate limiting surface film will primarily depend on the thickness of the surface film and the total surface area of the sample matrix.

The final step depicted in Fig. 8 is the transport of the analyte in the bulk fluid phase. Such transport is governed primarily by the diffusional coefficient of the analyte in the fluid medium. As noted previously, the diffusion coefficients of solutes in supercritical fluids are intermediate between those that they exhibit in liquid or gaseous media. This factor is independent of the sample matrix. In some instances, enhancement of the mass transfer of an analyte may be expected from free convection effects due to the variable extraction density of the supercritical fluid [94]. Such an effect is readily observed when conducting an extraction in a vertically-orientated cell, where an analyte concentration gradient exists due to the differences in the respective densities of extraction fluid and target analyte.

3.7 Problems and Future Research Needs in Analytical SFE

The successes of analytical SFE have been noted in the previous sections and amply demonstrated in the literature. However, as with any evolving technique, analytical SFE has capabilities and limitations that are not totally understood. Compared to liquid extractions, SFE has several more experimental factors that must be controlled and understood to achieve reproducible results. Some of these parameters are tabulated in Table 3 and have been discussed in previous sections.

Factors such as the collection technique and sample size are determined by the physical nature of the expected extract and sample homogeneity, respectively. Obviously, an extract consisting of volatiles cannot be collected by employing a simple phase separation. In this case, a packed accumulator cartridge would be the preferred collection device. Small samples should also be avoided in cases where the sample matrix is not homogeneous, since

Table 3. Experimental factors affecting analytical SFE

Pressure
Temperature
Flow rate
Extraction time
Collection technique
Sample size
Choice of supercritical fluid
Choice of modifier
Amount of modifier
System leaks
System contamination
Sample matrix

Table 4. Reproducibility of analytical scale supercritical fluid extractions

Analyte	Matrix	Concentration range (ppm)	% RSD[a]
Polyaromatic Hydrocarbons (PAH)	Diesel exhaust Particulate (NIST)	1.4 – 55	0.3 – 7.0
PAH	Urban dust (NIST)	2.0 – 8.2	0.5 – 1.0
PAH	Urban dust (NIST)	2.1 – 8.1	0.4 – 1.0
PAH	Tenax	0.1 – 2.0	3
PAH	River sediment	–	2 – 20
PAH	Lampblack	–	2.4 – 16
Dioxin	Soil	0.0016 – 0.0082	1 – 20
Spice components	Basil	–	6 – 17
Polymer additives	Polyethylene	5.5 – 1300	4 – 29
Menadione	Rodent feed	20 – 1500	0.4 – 4.7

[a] Percent relative standard deviation.

the resultant extract may not accurately reflect the content of the sample. Utilization of larger sample sizes in analytical SFE obviously favors off-line techniques, since on-line methodology is ultimately limited by the analyte concentrations that can be chromatographed.

The set of parameters listed in Table 3 makes optimization of analytical SFE to a particular analysis problem more time consuming than conventional extraction techniques. However, the application of statistical experimental design methods to SFE [95] promises to ease the burden on the analytical chemist. Excellent extraction reproducibility has been reported for a number of different sample types using SFE as shown in Table 4.

Coextraction of unwanted solutes along with the target analyte frequently occurs in analytical SFE, whether conducted in the off- or on-line mode. These interferences can be removed either by conventional sample cleanup methods or by utilizing a sorbent column downstream from the SFE device. Selective desorption of target analytes can be affected from coextracted background matrix components, provided there are sufficient differences in their respective breakthrough volumes on the sorbent in the presence of the supercritical fluid (see Fig. 6). Alternatively, one can use a selective detector which "blanks out" the interfering species in the analysis step. An example of this principle is shown in Fig. 9 where an on-line extraction has been performed on the pesticide, DDT, both neat and in a fat matrix with potentially interfering components. The use of an electron capture detector (ECD) after SFE and capillary SFC shows high specificity for the chlorinated pesticide, whereas a flame ionization detector (FID) trace on the same extract shows only a large unresolved triglyceride profile.

Finally, the opportunities for applying SFE in analytical chemistry are numerous and appear to be only partially offset by the above limitations. Routine use of the technique will require the development of instrumentation that will allow the analysis of multiple samples in either a batch or

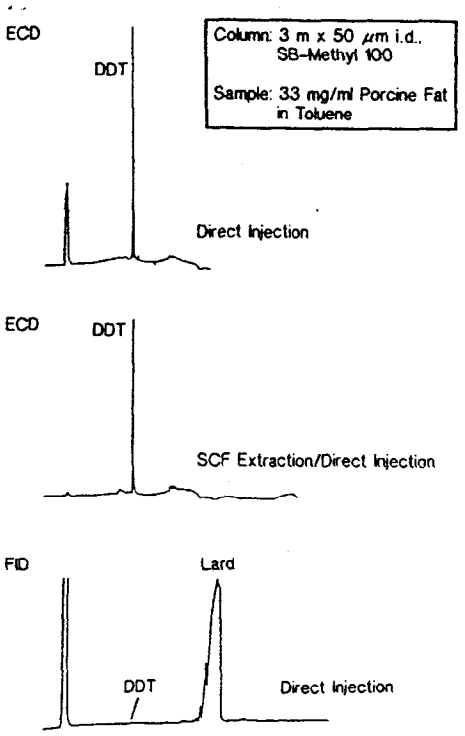


Fig. 9. Detector specificity for DDT-spiked pork fat by SFC and SFE-SFC

serial mode. These and other developments portend a promising future for SFE: a technique which will help the analytical chemist and improve his laboratory environment.

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